Claims

1. A method of simultaneously amplifying a plurality of target sequences within sample nucleic acid which comprises:

(a) contacting said sample nucleic acid with one or more primer pairs under conditions which allow hybridisation of the primers to the sample nucleic acid, each primer having a bipartite structure A-B wherein part A is specific for a particular target sequence within the sample nucleic acid and part B is a constant sequence which is common to all primers or is common amongst all forward primers with a different sequence common amongst all reverse primers;

(b) performing a first amplification reaction;

(c) degrading the bipartite primers or separating them from the amplification products of the first amplification reaction;

(d) contacting the amplification products from the first amplification reaction with primers which comprise part B of the bipartite primers or a nucleotide sequence which is substantially identical to part B, under conditions which allow hybridisation of the primers to the amplification products; and

(e) performing a second amplification reaction.

2. A method as claimed in claim 1 wherein the constant region B of the bipartite primers is common between both forward and reverse primers.

3. A method as claimed in claim 1 or claim 2 wherein the constant region B is 10-40 nucleotides in length.

4. A method as claimed in any preceding claim wherein the first amplification reaction comprises no more than 25 amplification cycles.

5. A method as claimed in any preceding claim wherein step (c) comprises contacting the bipartite primers with a DNA-modifying enzyme so as to cause degradation thereof.

6. A method as claimed in claim 5 wherein step (c) comprises contacting the bipartite primers with an exonuclease so as to cause degradation thereof.

7. A method as claimed in claim 5 wherein the bipartite primers contain one or more uracil residues.

8. A method as claimed in claim 7 wherein the modifying enzyme is uracil DNA glycosylase.

9. A method as claimed in claim 7 or 8 wherein the bipartite primers contain no thymine residues.

10. A method as claimed in any one of claims 7-9 wherein the bipartite primers contain uracil in part A but not part B.

11. A method as claimed in any one of claims 1-4 wherein step (c) comprises isolating the amplification products from the initial reaction mixture.

12. A method as claimed in claim 11 wherein the amplification products of the first amplification reaction are captured on a solid support.

13. A method as claimed in claim 12 wherein the amplification products are contacted with a probe incorporating a binding partner for a binding moiety provided on said solid support.

14. A method as claimed in any preceding claim wherein all of steps (a)-(e) are performed in one reaction

vessel.

15. A method as claimed in any preceding claim wherein 4 or more target sequences are amplified simultaneously.

16. A method as claimed in any preceding claim wherein one or more of the target sequence comprises a non-naturally occurring nucleotide sequence.

17. A method as claimed in claim 16 wherein the target sequence comprises regions which are not naturally found in juxtaposition.

18. A method as claimed in any preceding claim wherein one or more of the primer pairs is designed to hybridise either side of a junction region between a regulatory region and a coding region within sample nucleic acid.

19. A method as claimed in any preceding claim wherein the sample nucleic acid comprises host organism nucleic acid and a genetically engineered construct.

20. A method as claimed in claim 19 wherein one or more of the target sequences spans a region which comprises both host organism nucleic acid and inserted nucleic acid from the genetically engineered construct.

21. A method as claimed in any preceding claim wherein the products of the second amplification reaction are contacted with a plurality of different probes designed to hybridise to the target sequences under conditions which allow hybridisation thereof.

22. A method as claimed in claim 21 wherein the probes which hybridise to the target sequences are labelled at their 3' end.

23. A method as claimed in claim 22 wherein the labelled probes are captured on a solid support.

24. A method as claimed in any preceding claim wherein a known concentration of a control nucleic acid sequence is added to the sample nucleic acid prior to the first amplification reaction.

25. A method as claimed in claim 20 wherein a host species specific sequence is co-amplified with said target sequence which spans a region which comprises both host organism nucleic acid and inserted nucleic acid from the genetically engineered construct.

26. A kit for use in a method of nucleic acid amplification which comprises:

(a) a plurality of bipartite primer pairs of form A-B as defined in claim 1;
(b) means for degrading the bipartite primers or for separating them from the amplification products of a first amplification reaction; and optionally
(c) primers which comprise part B of the bipartite primers of component (a) or a nucleotide sequence which is substantially identical to part B of said primers.